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ACTIVITY OF SUPEROXIDE DISMUTASE ENZYME IN EARLY AND ADVANCED STAGES OF NASOPHARYNGEAL CARCINOMA

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Abstract

Introduction: Nasopharyngeal cancer (NPC) ranks-fourth among all cancers in Indonesia.
Objective: This study aimed to determine the levels of SOD in patients with NPC.
Method: This was a cross sectional study of analytic observational, using consecutive sampling, with 45 patients with NPC and 15 controls.
Result: Average of SOD enzyme levels were higher in the NPC patient group (±1877.87224 1137.119495) compared with the control group (±441.42120 320.355669) (p <0.05). SOD enzyme levels were higher in advanced stage±2060.67363 1179.147923 compared with early stage ± 1338.86685 680.958439 (p <0.05)
Conclusion: The level of SOD enzyme in patients with nasopharyngeal cancer is higher than in the control group, and the level is higher in the advanced stage group than in the early stage group.

Article Info

Keywords:
Nasopharyngeal cancer, superoxide dismutase enzyme, reactive oxygen species, carcinogenesis

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1. INTRODUCTION

1.1 Nasopharyngeal Carcinoma

Nasopharyngeal cancer (NPC) is a malignancy of squamous cells derived from epithelial lining of the nasopharynx. Globally, it is estimated of 65,000 new cases and 38,000 deaths per year [1]. Geographically, the highest incidence was in South China, especially in Guangdong province, with an incidence of 10 to 150 per 100,000 population per year, with an average age of 40-50 years [2], as well as in Southeast Asia, including Indonesia.

In Indonesia, NPC is the 4th most common malignancy, after cervix, breast, and skin. With incidence approximately 6.2 per 100,000 population. However, NPC is the 1st most common malignancy on entire head and neck malignancy in Indonesia, with comparison between men and women is 2-3: 1[3].

Actually, most patients are diagnosed with NPC during the symptomatic phase, because NPC showed some non spesific early symptoms and are often diagnosed at an advanced stage, where less than 1% of patients diagnosed at asymptomatic phase, for example, occurred when do imaging for other indications or when Epstein-Barr Virus serology detected in health screening. Early symptomps are not specific like a tinnitus, bloody nasal discharge, nasal congestion, chronic headaches, and even no symptomps in its early stages. It causes about 60-95% of NPC patients are diagnosed at advanced stage (III or IV) [4] . The initial diagnosis of NPC is very important for the succession of the treatment which is very dependent on the severity of the disease. The prognosis of patients with NPC clinical staging relies heavily on the time of diagnosis; where more than 80% of therapeutic efficacy occurred at early stage (stage I-II) and less than 40% success can be expected if patients are diagnosed at advanced stage (stage III-IV) [5].

1.2 Reactive Oxygen Species

Some of the endogenous cellular processes can lead to gene mutation which is the cause of the occurrence of some certain types of tumors in humans. Many reactions have been known to cause damage to DNA and play a role in the occurrence of spontaneous mutagenesis [6]. Reactive Oxygen Species (ROS) or free radicals have been shown to have a role in the process of carcinogenesis [7]. DNA damage caused by free radicals occur from a spontaneous mutation in mitochondria and nucleus genome [6]. Oxidative damage of the major cellular components such as DNA, lipids, carbohydrates and proteins associated with the phenomenon of aging, ischemia, cancer, autoimmune disease and degeneration of nerve cells [8]

The main free radicals resulting from the metabolism of oxygen to the body's biological systems are superoxide anion (O_2) produced through several important biophysiological systems in the body, including the activation and mitochondrial phagocytosis [9]. Superoxide products important in bactericidal activity but in an attempt to kill the bacteria it can cause tissue damage, depending on the amount and location of where its produced.

1.3 Superoxide Dismutase Enzyme

Endogenous antioxidant enzymes, Superoxide Dismutase enzme (SOD) together with myeloperoxidase, catalase and glutathione peroxidase are the backbone of prevention in the face of major cellular ROS [10]. ROS, including superoxide anion free radicals such as (O2-) and hydroxyl radicals (OH) as well as nonradical components such as hydrogen peroxidase (H2O2) is continuously produced by the body [11].

Under normal conditions, the cells are protected from the effects of high toxic concentrations of ROS levels through the balance of endogenous enzymatic antioxidant and nonenzymatic antioxidants [10]. However, oxidative damage can be caused by excessive production of ROS or imbalance of the body's defense mechanisms by antioxidants, and this has been associated with the development and treatment of a number of diseases such as cancer [11].

Superoxide dismutase is the first defense mechanism in the fight against free radicals, or oxidative damage that occurs spontaneously in cell [12, 13]. Copper/Zinc SOD (Cu/Zn SOD) is located in the cytoplasm and manganese SOD (MnSOD) is located in the nucleus [14].

2. MATERIAL AND METHODS

2.1 Patient Preparation

Forty five histopathologically proved cases of NPC, 29 males and 16 females (age 30-65 Years), attending the Department of ENT, Wahidin

Sudirohusodo Hospital, Makassar, were selected for the study. Results were compared with a group of fifteen normal healthy as control group, age and sex matched volunteers. Patients with cancer of any other regions who have already received treatment in any form and those having acute infections, hepatobiliary diseases, respiratory diseases or renal diseases were excluded.

Each patient was subjected to detailed clinical history, general physical examination, ENT examination including nasoendoscopy and examination of lymph nodes draining the areas. Radiological examination included nasopharyngeal CT-Scan and routine chest X-Ray. All the patients were also subjected to routine laboratory investigation.

2.2 Plasma Preparation

Three ml blood was collected into heparinised tubes from 60 patients according to a protocol approved by the Institutional. Human Review Board for the Protection of Human Subjects. Citrate–blood was centrifuged at 5000 RPM, the clear plasma was collected.

2.3 Superoxide Dismutase Enzyme Activity Determination

Superoxide Dismutase Enzyme activity was determined in 60 samples by spectrophotometry (420 nm) using sandwich ELISA. This research used catalog number LS-F5770, a product from LifeSpan BioSciences, Inc, North America.

2.4 Statistical Analysis

Statistical analysis was carried out by Student's t-test value, to assess the statistical significance of the obtained differences of SOD levels between NPC group and normal healthy group, and SOD levels between early (stage I and II) and advanced stages (stage III and IV) of NPC . A P value <0.05 was considered to be statistically significant.

3. RESULT

3.1 NPC Patients Characteristics

On table 1, majority of the NPC patients are 4th and 5th decades. And 64.4% of the patients are male, with 35.6% female. Majority of the patients have a lump in the neck as a primary complaint to seek medical profesional (40%). And mostly the patients were diagnosed in advanced stages (53.3%).

Table 1. NPC Patient Characteristics

Characteristics	Sum (n=45)	Percentage (%)
Age		
30-39	13	28.9
40-49	15	33.3
50-59	12	26.7
60-65	5	11.1
Sex		
Male	29	64.4
Female	16	35.6
Primary Complaint		
Bloodstained Discharge	6	10.0
Lump in the Neck	18	30.0
Epistaxis	8	13.3
Nasal Congestion	5	8.3
Chronic Headache	7	11.7
Tinnitus	1	1.7
Staging / TNM		
1	4	8.9
2	9	20.0
3	11	24.4
4	21	46.7
Stage		
Early	13	28.9
Advanced	32	71.1

3.2 Evaluation of SOD Activity

On table 2, there was a significantly difference of SOD activity between two groups, SOD activity was higher in NPC group (1877.87±1137.12 pg/ml) compared to in control group (441.42±320.35 pg/ml) (p<0.05).

Table 2. Plasma SOD, in NPC patient and control (pg/ml)

Group	n=60	Mean	SD	P
NPC	45	1877.87	1137.12	0.00
Control	15	441.42	320.35	

On table 3, there was a significantly difference of SOD activity between early and advanced stages, SOD activity was higher in advanced stages group (2060.67±1179.14 pg/ml) compared to early stages group (1338.87±680.96 pg/ml).

Table 3. Plasma SOD, in Early and Advanced Stages (pg/ml)

Group	n=60	Mean	SD	P
Eaky	13	1338.87	680.96	0.04
Advanced	32	2060.67	1179.14	

4. DISCUSSION

In this study, the majority of NPC patients aged on 4th and 5th, which is 70% of the total samples, similar to the characteristics of the study conducted by Cheng in 2001 [2], this could be caused by the aging process, people will be increasingly exposed to free radical and oxidative stress on the individual is higher, thus increasing the risk of cancer.

The majority of the samples in this study was male, with 71.1% of total samples with comparative for the male: female was 1.8: 1. This characteristic according to research conducted by Kuhuwael in 2001 in Makassar as well as by Susworo in 2004 in Yogyakarta [15, 16]. It can be caused by several environmental factors, one of which, the male tend more often to work outdoor, so it is more exposed to wood dust and smoke that became one of the predisposing factors of NPC. Also, lifestyle habits such as smoking cigarettes or alcohol consumption are higher in men compared to women.

Most of the primary complaints of patients when they comes to the hospital is a lump in the neck, which is about 30%, followed by epistaxis and bloodstained discharge of 23.3%, while that came with complaint of tinnitus is only 1.7%. This shows that the majority of patients come when the symptoms showed advanced stage, which after its regional metastasis to the neck lymphnodes, also the erosion of the nasopharynx, it is according to research conducted by Wei in 2006 in South China [17]. From the results of this study is found that about 71.1% of patients diagnosed at an advanced stage, which amounted to 24.4% in stage III and 46.7% in stage IV and only a small percentage are diagnosed at an early stage, ie 8.9% in stage I and 20% in stage II, which is in line with research by Soewito in 2009, of which nearly 60-95% of cases are diagnosed at stage III and IV [4]. This condition can be caused by NPC early symptoms are not specific and not typical, so it makes people do not seek medical profesional when the disease is still in early stage.

The main free radical that result from oxygen metabolism in the body's biological system are superoxide anions (O2-). Superoxide products are important in bactericidal activity, but in its effort, it can cause tissue damage, such as causing protein degradation, damage to cell membranes due to lipid oxidation, DNA damage, and oxidation of low density lipoproteins that cause genome instability and phenotypic mutations in some tumors. [10]. Superoxide Dismutase Enzyme (SOD) functions are to convert superoxide (O2-) to hydrogen peroxide (H2O2). Hydrogen peroxide thus formed is then converted to water (H2O) by catalase or by glutathione peroxidase. [14]

In the studies we conducted, the correlation was statistically significant (P <0.05) between the levels of SOD to NPC with average levels of 1877.87 pg/mL and standard deviations 1137.12, higher than in the control group, which was 441.42 pg/mL and standard deviations 320.36. It is demonstrated higher activity of SOD enzyme against oxidative stress response in patients with NPC. This situation illustrates the process of oxidative damage to DNA from a significant cause of the instability of the genome in cancer that affects humans, so the body produces SOD enzymes as the backbone of primary prevention to face the cellular oxidative stress.

Likewise, it was obtained statistically significant difference (P <0.05) in relation to the disease stages, the levels of SOD was higher at advanced stages ie stage III and IV, with average levels of 2060.67 pg/ml with a standard deviation of 1179.15, and at early stage that stage I and II, the average level was 1338.87 pg/ml with a standard deviation of 680.96. This shows that the more advanced stages of the NPC, the higher oxidative stress in the disease. This is in line with research relationship SOD and NPC conducted by Liu in 2012 and Salzman in 2007, where the levels of SOD increases ranging from early non-invasive stage, until more advanced metastatic stage where in theory there is excessive expression of SOD in metastatic disease, which is associated with SOD enzyme's ability to

catalyze the toxic O2 into H2O2, namely reactive oxygen species (ROS), which play a role in oxidative stress [11, 18].

5. CONCLUSION

The superoxide dismutase enzyme level were higher in patients with nasopharyngeal cancer compared to normal individuals. Level of the superoxide dismutase enzyme was higher in advanced stage of nasopharyngeal cancer (stage III and IV).

REFERENCE

[1] Chan J, F B, McCarron P. Patology & Genetics Head and Neck Tumour, WHO Classification of Tumours. Barnes L, editor. Lyon: IARCPress; 2005. 85-7 p.

[2] Cheng H. Nasopharyngeal Cancer and the Southeast Asian Patient2001; 63,1776-82:[1776-82 pp.].

[3] Punagi AQ, Eka S. Polimorfisme Gen VEGF Pada Gambaran Klinis Dan Histopatogi Karsinoma Nasofaring. Makassar: Fakultas Kedokteran Universitas Hasanuddin; 2007.

[4] Soewito M, Yati; Abdul, Kadir; Eka, Savitri; Burhanuddin, Bahar. Respon Antibodi IgA (VCA-p18 + EBNA 1) Terhadap EPSTEIN-BARR VIRUS (EBV) Pada Keluarga Penderita Kanker Nasofaring di Makassar. Tesis Program Pascasarjana Fakultas Kedokteran Universitas Hasanuddin. 2009.

[5] Kwong D, L, W; J, S, T, Sham; Au, G, K, H. Concurrent and Adjuvant Chemoterapy for Nasopharyngeal Carcinoma: A Factorial Study. Journal of Clinical Oncology. 2004;22:2643-53.

[6] Pol JAF, Douglas MG. Molecular interactions of cancer and age. Cancer in the elderly. . Hematol Oncol Clin North Am. 2000.

[7] Gargouri B. High levels of autoantibodies against catalase and superoxide dismutase in nasopharyngeal carcinoma. South Med J. 2009;102:1222–1226.

[8] Gros L, Saparbaev Mk, Laval J. Enzymology of the Repair of Free Radicals-Induced DNA Damage.. Review Oncogene 21(58), 8905-25. 2002.

[9] Klaunig J, E; M, Lisa, Kamendulis; Barbara, A, Hocevar. Oxidative Stress and Oxidative Damage in Carcinogenesis. Toxicologic Pathology. 2010;38:96-109.

[10] Kinnula VL, Crapo JD. Superoxide Dismutases in Malignant Cells and Human Tumors Free Radical Biology & Medicine, 36, 718-744. 2004.

[11] Liu J, Zhan X, Li M, Guoqing L, Pengfei Z. Mitochondrial proteomics of nasopharyngeal carcinoma metastasis. BMC Medical Genomics. 2012;5:62.

[12] Hileman Ea, Achanta G, Huang P. Superoxide dismutase: an emerging target for cancer therapeutics. . Expert Opinion on Therapeutic Targets, 5, 697-710. 2001.

[13] Cerutti P, Ghosh R, Oya Y, Amstad P. The Role of the Cellular Antioxidant Defense in Oxidant Carcinogenesis. . Enviromental Health Perspectives, 102. 1994.

[14] Borrelli A, Schiattarella A, Mancini R, Morrica B. A recombinant MnSOD is radioprotective for normal cells and radiosensitizing for tumor cells. Free Radical Biology & Medicine, 46, 110-116. 2009.

[15] Kuhuwael FG. Aspek klinis karsinoma nasofaring di RSU Dadi dan RS.Wahidin Sudirohusodo tahun 1990-1999. Fakultas Kedokteran UNHAS 2001.

[16] Susworo R. Kanker Nasofaring Epidemiologi dan Pengobatan Mutakhir. Cermin Dunia Kedokteran. 2004;144,16-18.

[17] Wei W, I. . Nasopharyngeal Cancer. Philadelphia: Lippincott Williams & Wilkins; 2006.

[18] Salzman R, Kankova K, Pacal L. Increased activity of superoxide dismutase in advanced stages of head and neck squamous cell carcinoma with locoregional metastases. Neoplasma. 2007;54(4):321-5.